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ANTIMICROBIAL SUSCEPTIBILITY OF UROPATHOGENIC CLINICAL ISOLATES of *Escherichia coli*

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ABSTRACT
Urinary tract infections (UTI) are infectious diseases common in women and it is estimated a global spending about 6 billion dollars on health care related to its morbidity. This study evaluated the *in vitro* susceptibility of clinical uropathogenic isolates from *E. coli* to Meropenem, Cephadroxiladroxil and Ceftriaxone. Broth Microdilution assays were performed to determinate the minimum bactericidal concentration (MBC) of clinical isolates, with sensitivity of all strains to the tested concentrations of Meropenem and Ceftriaxone, and a varied profile of resistance to Cephadroxil. Our data suggest the need for pharmacovigilance monitoring and contribute to the epidemiological study of local conditions of resistance to these drugs.
Key Words: Urinary tract infections, *E. coli*, Antimicrobials.

Introduction

Urinary tract infections (UTI) are infectious diseases common in women and generate significant costs to the public purse. It is estimated to be annually spent about 6 billion dollars worldwide on health care related to its morbidity[1]. The most common pathogens associated with UTI are those belonging to the family *Enterobacteriaceae* and include *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter*, *Proteus mirabilis* and *Entobacter sp*, being *E. coli* the more frequent pathogen[2,3]. Recent data showed that the *E. coli* was responsible for 44.4% of UTI in a sample of Brazilian patients[4]. A similar value was described in the United States, with 48.9% prevalence in the same type of infection[5].

E. coli is a gram-negative, anaerobic bacterium optional and an emerging enteropathogen, especially in developing countries. Most clinical samples is in the intestinal microbiota, however, approximately 10% are pathogenic[6,7]. Currently, six virulent variants of *E. coli* are known, being the enteropathogenic (EPEC) responsible for around 90% of urinary tract infections[1].

The therapeutic scheme of UTI is planned empirically using often broad-spectrum antimicrobials. The treatment of hospitalized patients with complicated infections has become more difficult due to the rapid increase of microbial resistance to major drugs employed in clinical practice[5]. It is relevant, therefore, that an antimicrobial susceptibility study contributes to epidemiological surveillance and to point paths to the use of alternative drugs.

The objective of this study was to evaluate the *in vitro* susceptibility of clinical uropathogenic *E. coli* isolates to meropenem, a broad-spectrum carbapenem drug, and also to the cephadroxiladroxil and ceftriaxone, first and third generation cephadroxilalosporins, respectively. Through broth microdilution assays, we determined the minimum bactericidal concentration (MBC)

of clinical isolates. Our data indicated sensitivity to meropenem and ceftriaxone, with varied profile of resistance to cephadroxiladroxil, and contributes to a better knowledge of the local conditions of resistance to these drugs.

Materials and Methods

For antimicrobial susceptibility testing, ten uropathogenic clinical isolates of *E. coli* from the collection of the Microbiology Laboratory at University Vale do Rio Doce, with an ATCC strain 25922. Microorganisms were properly identified by Vitek 2 automated system (version RO4.O2, bioMérieux), according to the manufacturer's instructions. A gram-negative identification card (ID-GNB) was inoculated with a bacterial suspension prepared in 0.9% saline equal to the turbidity of a 0.5 McFarland standard. Discrepant bacterial identifications were resolved by retesting the isolates and by reference biochemical tests. For assays, strains were prepared on Mueller-Hinton broth with cellular concentration of 0.5 in McFarland turbidity scale ($\approx$ 1, 5 x 10^5 cfu/mL).

The tests for the determination of the MBC were performed in non-treated polystyrene plates, followed by the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2010) with modifications. For assays, we used intravenous formulations of hospital use of Meropenem (500 mg, Astrazeneca, UK), Ceftriaxone (500 mg, Roche, USA), and Cephadroxil (Bristol Myers Squibb, USA), reconstituted with 0.9% sterile saline solution as recommended by the manufacturer. Antimicrobial tests were made separately with each formulation preservative systems, prepared with the same saline solution, where we observed no effectiveness against the microorganisms of this study.

Results

The bacterial growth observed in the tests is shown in the following tables. Table 1 represents the results obtained with meropenem and table 2 represents the results with ceftriaxone. All isolates, but the ATCC strain 25922, were susceptible to all concentrations tested in both drugs. The MBC considered for meropenem was 0.39 mg/mL and the MBC considered to ceftriaxone was 0.78 mg/mL.

E. coli Strain	Meropenem concentration (mg/mL)						
	25.0	12.5	6.25	3.12	1.56	0.78	0.39
E1	-	-	-	-	-	-	-
E2	-	-	-	-	-	-	-
E3	-	-	-	-	-	-	-
E4	-	-	-	-	-	-	-
E5	-	-	-	-	-	-	-
E6	-	-	-	-	-	-	-
E7	-	-	-	-	-	-	-
E8	-	-	-	-	-	-	-
E9	-	-	-	-	-	-	-
E10	-	-	-	-	-	-	-
ATCC25922	-	-	-	-	-	-	+

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Table 1: MBC of Meropenem for clinical isolates of *Escherichia coli.*

E. coli Strain	Ceftriaxone concentration (mg/mL)						
	50.0	25.0	12.5	6.25	3.12	1.56	0.78
E1	-	-	-	-	-	-	-
E2	-	-	-	-	-	-	-
E3	-	-	-	-	-	-	-
E4	-	-	-	-	-	-	-
E5	-	-	-	-	-	-	-
E6	-	-	-	-	-	-	-
E7	-	-	-	-	-	-	-
E8	-	-	-	-	-	-	-
E9	-	-	-	-	-	-	-
E10	-	-	-	-	-	-	-
ATCC25922	-	-	-	-	+	-	-

Table 2: MBC of Ceftriaxone for clinical isolates of *Escherichia coli.*

Table 3 registers the results obtained when susceptibility to cephadroxil was assessed. The MBC ranged differently among the strains, and no concentration value could be established for all strains. However, the dose of 37.5 mg/mL was the most effective for the largest number of strains. Most of the strains (E1, E2, E4, E5, E6, E9, E10 and ATCC25922) were resistant in lower concentrations.

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E. coli Strain	Cephadroxil concentration (mg/mL)						
	75.0	37.5	18.75	9.37	4.68	2.34	1.17
E1	+	+	+	+	+	+	+
E2	-	-	+	+	+	+	+
E3	-	-	-	-	+	+	+
E4	-	-	+	+	+	+	+
E5	-	-	+	+	+	+	+
E6	-	-	+	+	+	+	+
E7	-	-	-	-	+	+	+
E8	-	-	-	+	+	+	+
E9	-	-	+	+	+	+	+
E10	+	+	+	+	+	+	+
ATCC25922	+	+	+	+	+	+	+

Table 3: MBC of Cephadroxil for the clinical isolates of *Escherichia coli.*

Discussion

The highest levels of susceptibility were observed for meropenem and ceftriaxone. Similar results were found by Hawser et al. (2013): the *E. coli* strains showed 100% sensitivity for the carbapenems and of 89-91% for cephalosporins. Carbapenems are broad spectrum drugs and provide coverage even against anaerobic bacteria[9]. Bouchillon et al. (2013) suggests the use of carbapenems as an alternative antimicrobial treatment, which although are not first-line treatment drugs, they cannot be ignored in clinical practice. In this same study, it was demonstrated susceptibility of 90% to cephalosporins in gram-negative strains not producing extended spectrum β-lactamases.

Cephadroxil was the antimicrobial drug that showed lower susceptibility in this study, with a MBC of 37.5 mg/mL. This result is consistent with others regarding this drug[3], and a possible explanation for such result is the availability of this drug for oral administration by patients, whom may use the medication incorrectly.

Bacterial resistance is critical public health problem. The selection of resistant microorganisms is rising relatively fast while the rate of introduction of new drugs is slow[9]. The empirical therapeutic scheme employed when considering intravenous administration in hospitalized patients generally consists of sulfamethoxazole-trimetropim, amoxicillin combined to potassium clavulanate, ampicillin-sulfactam, aztreonam, gentamicin, amikacin and, less frequently, and in cases of sepsis by urinary focus, meropenem[2,9,10]. An antimicrobial indicated empirically must be replaced or added to another when the rate of local antimicrobial resistance exceeds 20%[2,4]. There are compelling evidences of rising resistance to the main antimicrobials used in clinical practice, mostly regarding sulmetozazol-trimetropim, ampicillin and, more recently, the first and second generation cephalosporins, limiting the use of these agents as first-choice antimicrobial drugs[3,4,5,11].

The present study demonstrated that although they have been reported several cases of resistance to carbapenems due to carbapenases in recent years, these agents have maintained a high level of activity against the

uropathogenic isolates of *E. coli* . Our data is consistent with the observation of others when analyzing susceptibility profiles of clinical isolates of *E. coli in* vitro [5,8,12,13,14] .

The third-generation cephalosporins are resistant to many β-lactamases and are active against *Enterobacteria*[3,9]. However, the literature reports increase in occurrence of resistant strains, estimating a limited future use and suggesting constant surveillance of resistance levels, which rose from 2.7% in 2003 to 8.3% in 2009 in Europe[8,14]. The mechanism of resistance may be due to hyperexpression of β-lactamases associated with decreased permeability of outer membrane or hyperexpression of efflux pumps, which reduce the concentration of antimicrobial drugs on bacterial cells[15,17].

Conclusion

Although the production of β-lactamases and carbapenases strains by *E. coli* strains have been growing in recent years, meropenem and ceftriaxone were effective against the clinical isolates of this study. The use of these drugs requires a continuous monitoring of effectiveness through programs of pharmacovigilance, as empirical therapy is often ineffective due to antimicrobial resistance, and often not directly by prescribing errors.

The evaluation of susceptibility profiles can be useful in developing strategies aiming the rational use of antimicrobials, and provide rationale for the empirical choice of a drug. Studies that describe specifically the susceptibility of uropathogens are relatively limited, however, the establishment of the antimicrobial sensitivity profile is of high relevance, because they can be significantly different even among near regions, once they are associated with local selective pressures that promote genetic variability in microorganisms, what may influence the sensitivity to different drugs[3,4].

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